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Elucidate the Role of Corepressors as Regulators of
Progesterone Receptor Transcriptional Activity

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13. ABSTRACT (Maximum 200 Words)

Progesterone regulates diverse aspects of proliferation, differentiation and development of mammary, uterine and nervous tissues. The biological functions of progesterone are mediated through the progesterone receptor (PR) whose transcriptional activities are modulated by corepressors and coactivators. We are interested in studying the interaction between corepressors and PR with a view of evaluating the role of this complex on the pharmacology of progestins and anti-progestins in breast cancer. Our group observed that corepressor N-CoR binds to antagonist-bound PR in cells and suppresses 8-bromo-cAMP mediated potentiation of PR transcriptional activity (Wagner et al., 1998). Over-expression of N-CoR represses the partial agonist activity of RU486-bound PR (Jackson et al., 1997). These observations highlight N-CoR as key regulators of PR pharmacology. In the first year of this research we undertook a phage display approach to identify the protein-protein interaction surfaces on N-CoR. Among the phage peptides associating with N-CoR, one peptide shared striking similarity to coactivator ACTR. This finding has subsequently lead to the discovery that corepressor N-CoR directly interacts with coactivator ACTR. Our research results suggested that transcriptional repression and activation, the two processes which both involved in breast cancer biology, are integrated in a manner that are not previously anticipated. We will next test how corepressors and coactivators coordinate in regulating progesterone action in normal and breast cancer cells.

14. SUBJECT TERMS
Research area: nuclear receptors, corepressors, N-CoR, progesterone, receptor, anti-progestin, technical description: phage display, peptides, inhibitors

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Table of Contents

Introduction

The major goal of this research is to study the mechanism of breast cancer biology by defining the roles of corepressor and coactivator in coordinating progesterone receptor (PR) action. PR is implicated in the pathology of hormone-dependent human breast cancers, which has led to the use of progesterone antagonists as chemotherapeutics for the disease [1]. The transcriptional activities of PR and other nuclear receptors are modulated by corepressors and coactivators [2]. The first direct link between corepressor N-CoR and PR was provided by Jackson et al. when they identified N-CoR in a two hybrid screen using RU486/PR as a bait [3]. Subsequently our group observed that N-CoR binds to antagonist-bound PR in cells and suppresses 8-bromo-cAMP mediated potentiation of PR transcriptional activity [4]. The pharmacological significance of these observations was strengthened by the recent finding in mice that the transition of ER positive, tamoxifen sensitive, MCF-7 cell xenografts to a state of tamoxifen resistance is accompanied by a decrease in the expression of N-CoR [5]. Potential roles of coactivators in breast cells were highlighted by the finding that in the ER positive breast and ovarian cancer cells, the expression level of coactivator ACTR is significantly increased [6]. In the first year of this research we have undertaken a phage display approach to identify the protein-protein interaction surfaces on N-CoR in order to define the biochemical processes that modulate PR transcriptional activity. Among the phage peptides what associate N-CoR, one peptide shared striking similarity to ACTR. This finding has subsequently lead to the discovery that corepressor N-CoR directly interacts with coactivator ACTR. We observed that N-CoR contributes to transcriptional activation by recruiting ACTR to nuclear receptors prior to ligand activation. On the other hand, ACTR appears to partially reverse the transcriptional repression of N-CoR by interfering with N-CoR/HDAC4 interaction. These findings suggested that transcriptional repression and activation, the two processes which both involved in breast cancer biology, are integrated in a manner that are not previously anticipated.

Body

Identification of protein-protein interaction surfaces on N-CoR using phage display.

In the Specific Aim 1 of my research proposal, I planed to isolate phage peptides that bind to the Receptor Interacting Domains (IDs) of N-CoR. In the Statement Of Work, I estimated that this task would be done in Months 1-10. In the past 12 months this work has been completed and its biological significance has been evaluated as reported herein.

I undertook a phage display approach to identify the protein-protein interaction surfaces on N-CoR. I identified 24 phage peptides (7-16mers) that bind to the ID1 of N-CoR. Remarkably, one of these peptides, L1-7 contains significant sequence similarity to the nuclear receptor coactivator ACTR [6, 7]. Peptide L1-7 shares an identical 6-residue stretch (SHLGTK) with amino acids 832-837 of ACTR (Fig. 1). Additional peptides with less significant similarity to ACTR were also identified by colleagues in the lab. One of such peptide, L8-24, which binds to RD3 of N-CoR, is identical in 5 out of 8 residues to ACTR residues 424-431. Given that peptides L1-7 and L8-24 were (1) isolated from two different libraries and share no similarity to each other, (2) bind to two distinct domains of N-CoR, and (3) match two distinct regions of ACTR, we consider it likely that N-CoR and ACTR physically interact. In this study, we tested the possibility that N-CoR and ACTR interact with each other and further sought to elucidate the functional significance of this interaction with respect to transcriptional repression and activation.

N-CoR interacts directly with ACTR in vitro and in vivo.

We performed GST pull-down experiments to confirm that the N-CoR and ACTR proteins can interact directly. As shown in Figure 2A, RD3 and ID1, the two regions of N-CoR that associate with ACTR-like peptides, interact with full length *in vitro* translated ACTR. ACTR appears to bind more efficiently to a fragment of N-CoR that contains both ID1/ID2 (aa 1944-2453) than a fragment that contains ID1 alone. These results confirm that N-CoR interacts with ACTR and that RD3 and ID1/ID2 are involved in mediating this interaction.

Direct N-CoR/ACTR interaction was also observed in cells, with both transfected and endogenous proteins. In transfected 293T cells, we were able to show that ACTR and a myc-tagged fragment of N-CoR (aa 759-2453) could be co-immunoprecipitated, using an antibody against ACTR to IP and a myc antibody to detect the tagged $N-CoR_{759-2453}$ by western blot (Fig. 2B). Furthermore, we were able to show an interaction between endogenous full length N-CoR and endogenous full length ACTR by immunoprecipitating with an N-CoR antibody, and immunoblotting the pulled down fraction against ACTR. Consistent with the IP results, GST pull-down experiments (Fig. 3A) and mammalian two hybrid experiments (Fig. 3B) further support the hypothesis that N-CoR and ACTR directly interact. In the GST pull-down experiments, in vitro-translated full-length N-CoR and $N-CoR_{759-2453}$ can associate equally well with $ACTR_{400-1000}$. Likewise, in the mammalian two-hybrid assay, significant interaction between full-length versions of ACTR and N-CoR (Gal4-ACTR and VP16-N-CoR) was observed.

Next, we wanted to investigate whether the N-CoR/ACTR interaction applies to other coregulator proteins with homology to N-CoR or ACTR. A GST pull-down assay shown in Figure 3A suggests that SMRT, a corepressor protein homologous to N-CoR, also has the ability to bind ACTR quite well. However, the p160 coactivator proteins SRC-1 and GRIP1, which are homologous to ACTR, were only found to weakly bind N-CoR in a mammalian two-hybrid

5

assays (Fig. 3B). These results suggest that corepressor/coactivator interactions may not occur universally, but may occur in specific circumstances, depending on the complement of coregulators present.

Characterization of the interaction surface on N-CoR and ACTR.

Based on the studies so far, we concluded that an extensive surface on N-CoR, including at least RD3 and ID1 of N-CoR, contributes to the interaction with ACTR. A large fragment of N-CoR, N-CoR$_{759-2453}$, which encompasses RD3, ID1 and extended flanking region, display ACTR binding activity similar to full-length N-CoR (Fig. 3A). N-CoR$_{759-2453}$ has been employed as a derivative of full-length N-CoR in our study especially in the mammalian two-hybrid system. Lack of the N-terminus of the protein has make N-CoR$_{759-2453}$ to avoid the problems of proteasome-dependent degradation of N-CoR and Sin3a-mediated repression, both of which require the N-terminus of N-CoR [8, 9].

We next evaluated the surface on ACTR that is involved in its association with N-CoR. Although full-length ACTR displays interaction with N-CoR in the mammalian two-hybrid assay (Fig. 2C), the magnitude of the interaction in this assay is difficult to ascertain as ACTR exhibits a high level of basal transcriptional activity when tethered to DNA. To eliminate the confounding influence of ACTR's inherent transcriptional activity, we evaluated the interaction of VP16-N-CoR with ACTR$_{400-1000}$, a fragment of the coactivator which lacks the CBP binding sites and the intrinsic HAT activity that full length ACTR possesses within its C-terminus [7]. Using ACTR$_{400-1000}$ in this assay instead of full length ACTR reduced the high basal activity of the Gal4-coactivator. When assayed for its interaction with VP16-N-CoR$_{759-2453}$, Gal4-ACTR$_{400-1000}$'s total response is equivalent to that of full length ACTR, yet its fold of induction is much greater. This result indicates that ACTR$_{400-1000}$ contains the major N-CoR interaction surface. This was not surprising, however, as the sequences in ACTR that correspond to the peptides we identified in phage display are contained within ACTR$_{400-1000}$. Also included in $_{ACTR400-1000}$ is the receptor interaction domain or RID (aa 621-821) which is responsible for mediating the coactivator's interaction with nuclear receptors. Although ACTR$_{621-821}$ and ACTR$_{400-1000}$ display similarly strong TR binding activity in a hormone dependent manner (data not shown), ACTR$_{621-821}$ interacts with N-CoR much weaker than full-length ACTR or ACTR$_{400-1000}$ when tested in a mammalian two-hybrid assay. We conclude from these experiments that the surfaces within ACTR which interact with N-CoR are distinct from those required for TR binding, and that sequences in addition to those implicated by the L1-7 and L8-24 peptides are involved in N-CoR-ACTR interactions.

N-CoR, ACTR and TR form a trimeric complex in target cells.

Previous studies have shown that N-CoR and TRβ form a complex in the absence of hormone [8]. In this study we have shown that ACTR and TR do not interact in the same manner with N-CoR. The combination of these findings led us to hypothesize that N-CoR, ACTR and apo-TR may form a trimeric complex. This possibility was first tested *in vitro* using GST pull-down assays (Fig. 4A). As expected, we were able to demonstrate that TRβ binds to ACTR in a T3-dependent manner. Addition of N-CoR, however, resulted in significant ACTR/TRβ interaction in the absence of T3, suggesting that N-CoR can participate in a trimeric complex by possibly functioning as a bridge between TRβ and ACTR. In the presence of T3, ACTR and TRβ interact strongly, and under the conditions of this assay addition of N-CoR has no further potentiating effect.

Analogous results regarding the trimeric complex were observed using a three-hybrid assay (Fig. 4B), in which we were able to demonstrate that GAL4-ACTR and VP16-TRβ could interact in the absence of T3 only when N-CoR, which contains both the TR and ACTR binding sites, was co-expressed. To demonstrate that the binding between Gal4-ACTR and apo VP16-TRb depends on the bridging function of N-CoR and does not occur due to some inherent ability of apo VP16-TRβ to bind Gal4-ACTR, we also used two TR AF-2 mutants, L454R and E457K. It has been shown previously that the GRIP1 binding activity of L454R and E457K are abolished [10]. We observed that L454R and E457K retain N-CoR binding activity, but neither mutant receptor has the ability to bind ACTR, with or without thyroid hormone (data not shown). In our three-hybrid assay, we have found that coexpression of N-CoR allows the TR mutants L454R and E457K to interact with ACTR indirectly (Fig. 4B) and that this interaction is totally dependent on the presence of N-CoR. This finding strengthens our hypothesis that in the absence of thyroid hormone, TRβ and ACTR can interact with each other in an indirect manner, by simultaneously binding to N-CoR. Contrary to previous models of TRβ action, our experiments suggest that ACTR and N-CoR do not bind TR in a completely separate manner; in the apo state, both coregulators can exist in the same complex with TR.

Previous studies have shown that upon ligand activation of TRβ, the interaction between N-CoR and TRβ is lost and a strong interaction between liganded TRβ and a coactivator ensues. Therefore, we sought to determine the effect of ligand activated TRβ on the interaction between ACTR and N-CoR. As shown in Figure 4C, addition of ligand activated TRβ destabilizes the interaction between Gal4-ACTR and VP16-N-CoR$_{759-2453}$, whereas apo TRβ had no effect on the corepressor/coactivator interaction. We propose that formation of the liganded TR/ACTR complex interferes with the surface on ACTR that are required for N-CoR/ACTR interaction. Accordingly, liganded TR mutants that can not associate with ACTR should not be able to interfere with N-CoR/ACTR interaction. Indeed, figure 3C shows that the mutant TRs, L454R and E457K, can not disrupt N-CoR/ACTR interaction upon ligand activation. Overall, our experiments with wild type and mutant TRs suggest that N-CoR, ACTR and apo TRβ can form a trimeric complex, with N-CoR serving as the essential bridging factor between TR and ACTR. Furthermore, upon addition of hormone, the strong and direct interaction that occurs between TRβ and ACTR is responsible for actively ejecting N-CoR from the complex, destroying the corepressor's interaction with both the receptor and co-activator.

N-CoR potentiates transcriptional activation of TR/ACTR complex.

Our results thus far also suggest that in the absence of ligand, N-CoR functions not only to suppress TR-mediated gene expression but also to raise the local concentration of ACTR in such a way that agonist induced displacement of the corepressor is sufficient to initiate transcription. Such pre-recruitment of ACTR should enhance agonist-activated transcription. To test this hypothesis, we transiently transfected CV1 cells with a TRE-containing reporter plus vectors expressing TRβ, ACTR and N-CoR (Fig. 5A). Transfected cells were grown without hormone to allow the formation of the N-CoR/ACTR/TR complex. Subsequently, T3 was added to the cells and the TR-mediated transcriptional activity was measured following an additional period of incubation. In this cell system, we observed that TRβ was an effective activator of the TRE-containing reporter gene and that this activity was enhanced modestly by ACTR. Importantly, however, co-expression of N-CoR and ACTR, but not N-CoR alone, enhanced TRβ-mediated activation significantly. Western blot analysis was used to demonstrate that over-expression of N-CoR does not affect the expression level of either TRβ or ACTR (data not

shown). The basal activity of apo TR in the presence of ACTR and/or N-CoR showed no significant changes over TR alone (data not shown). It appears, therefore, that N-CoR increases the dynamic range of TR-transcriptional activity by enhancing its ability to suppress the basal activity of TRE-containing genes in the absence of hormone, while at the same time, recruiting ACTR to the promoter and facilitating a rapid and robust response to agonist activation.

Based on our findings and those of others we have developed a schematic model to describe how the N-CoR/ACTR/TR complex may be involved in the regulation of thyroid hormone action (Fig. 5B). We propose that in the absence of thyroid hormone, TRβ resides on a TRE within the promoter of target genes. The apo-receptor is then capable of recruiting the N-CoR/ACTR complex. Upon binding T3, TRβ undergoes a conformational change that abolishes the N-CoR/TRβ interaction and favors the association of ACTR with the TRβ coactivator-binding pocket. This strong interaction between liganded TRβ and ACTR appears to be involved in ejecting N-CoR from the complex. This model suggests that the processes of activation and repression are more closely linked than originally anticipated and provide a mechanism to explain the differences in cellular responses to thyroid hormone.

ACTR reverses N-CoR-mediated transcriptional repression.

One of the most surprising findings of our studies was that ACTR, a transcriptional coactivator, could interact with the RD3 domain of N-CoR, a domain that has previously been shown to possess the ability to repress transcription when tethered to DNA. Consequently, we considered the possibility that binding of ACTR to RD3 may modulate the repressor function of N-CoR. In agreement with these previous studies, we were able to show that the basal transcriptional activity of a GAL4-responsive reporter was suppressed by co-expression of GAL4-N-CoR (Fig. 6A). Interestingly, over-expression of full-length ACTR, or ACTR$_{400\text{-}1000}$ (which does not contain any activation functions), can reverse N-CoR-mediated transcriptional repression (Fig.6A). We verified that the ACTR$_{400\text{-}1000}$ derivative does not possess any co-activation function by showing that it was unable to potentiate the transcriptional activity of ligand activated TRβ (data not shown). Thus, ACTR may function both as a transcriptional activator when associated with ligand activated TR, and also as a modulator of the basal transcriptional activity exhibited by apo-TR.

Although the precise mechanism by which the N-CoR complex represses basal transcription has not yet been defined, recent studies have indicated that the histone deacetylases HDAC4, which directly associate with RD3, may be involved [11]. Our finding that ACTR binds to RD3 raises the possibility that HDAC4 and ACTR may compete with each other by recognizing overlapping regions within the RD3 of N-CoR. In support of this hypothesis we were able to show, using a GST pull-down assay, that RD3 of N-CoR interacts with HDAC4 and that this interaction is compromised by addition of *in vitro*-translated ACTR (Fig. 6B). This observation suggests that ACTR might interfere with TRβ-mediated repression by competing with HDAC4 (or a related protein) for binding to RD3 (Fig. 7C).

Figure 1

A

N-CoR

RD1   RD2   RD3   ID1   ID2

1   759   1944   2453

B

L1-7:   SSHLGTKFFSR

ACTR 832-837:   SHLGTK

L8-24:   SSRVMGLKDAYMYSR

ACTR 424-431:   SRAYGLAD

C

ACTR

                 RID   HAT

                              CBP binding

1   400   1000   1412
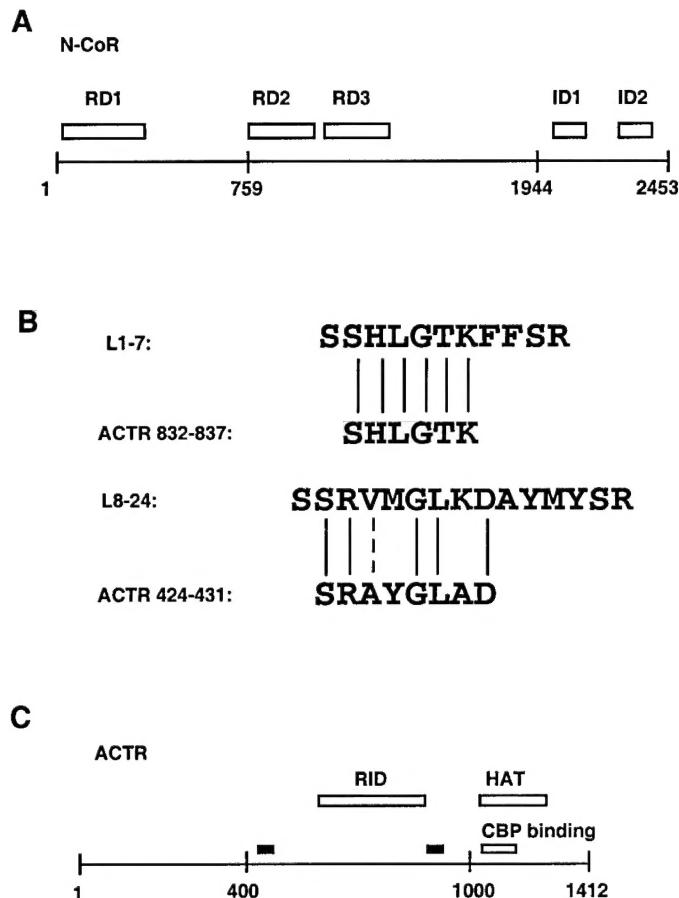
Figure 1. N-CoR-associated peptide contains sequence similar to ACTR.
(A) The domain structure of N-CoR. (B) N-CoR associated peptide L1-7 show ACTR-like sequence. We screened 7 combinatorial libraries using ID1 as targets. The peptide libraries contain random peptides ranging from 7-mer to 16-mer expressed in the M13-based vector mBAX. The procedure for phage was as previously described [12]. As a result, 24 ID1 binding peptides were recovered. The sequences of the peptides were subject to BLAST search at http://www.ncbi.nlm.gov/. (C) Domain structure of ACTR derived from a previous report [7]. The regions that correspond to the N-CoR binding peptides are labeled as solid bars. RID: receptor-interacting domain; HAT: histone acetyl transferase.

Figure 2

A

ACTR ▶

input   GST   RD3   ID1   ID1/ID2
                  |_____|
                      GST-N-CoR

B

293T, transfected

                    IP
input   IgG   ACTR

myc ▶

ACTR ▶

293T, endogenous

                    IP
input   IgG   N-CoR

ACTR ▶

N-CoR ▶

C

5Gal4Luc3

| VP16 | + | − | + | − | + | − | + | − |
| VP16-N-CoR759-2453 | − | + | − | + | − | + | − | + |
| | Gal4 | | Gal4-ACTR | | Gal4-ACTR400-1000 | | Gal4-ACTR621-821 | |
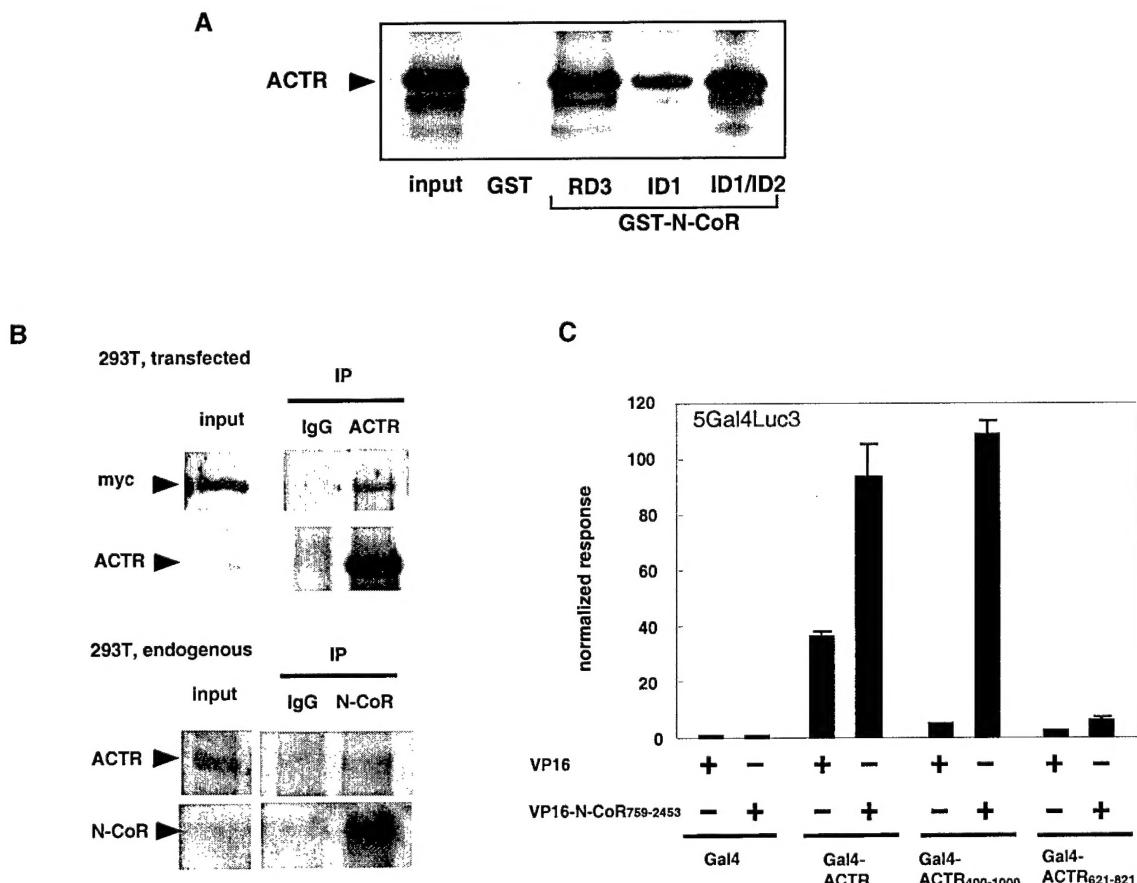
Figure 2. N-CoR and ACTR directly interact in vitro and in vivo.
(A) N-CoR domains associate with ACTR in GST pull-down experiments. N-CoR domains RD3 (aa 1017-1461), ID1 (aa 2063-2142), ID1/ID2 (aa 1944-2453) were expressed as GST fusion proteins and isolated using glutathione-conjugated beads. GST fusion or GST alone (5 μg each) was incubated with full-length 35S-ACTR. Input: 10%. (B) Co-immunoprecipitation between N-CoR and ACTR. 293T cells with 50% confluency were transfected with pcDNA3-ACTR and Myc-N-CoR759-2453 plasmids and were grown for additional 2 days. Whole cell lysis were immuno-precipitated by rabbit anti-ACTR and immuno-blotted by mouse anti-myc. The same membrane was re-blotted with rabbit anti-ACTR to confirm the IP results. For endogenous co-IP, whole cell extract of 293T cells or nuclear extract of CV1 cells were generated and were IP with goat anti-N-CoR. Immunoblots with rabbit anti-ACTR or goat anti-N-CoR were performed subsequently. (C) ACTR domains interact with N-CoR in the mammalian two-hybrid assay. cDNA fragments representing ACTR full length, aa 400-1000 and aa 621-821 were expressed as Gal4-DBD fusion proteins.

10
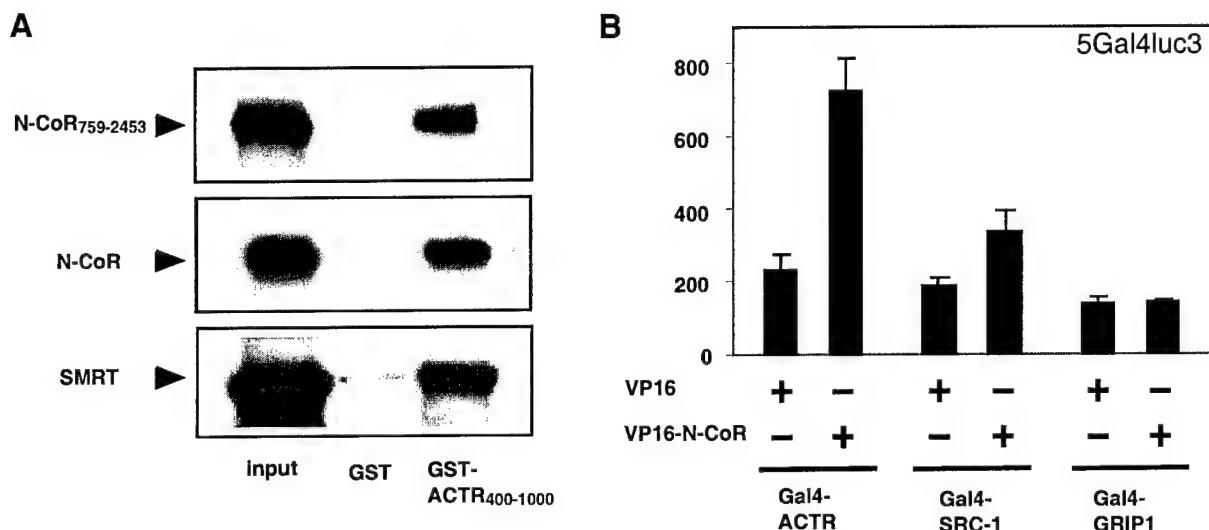
Figure 3

Figure 3. SMRT and p160 coactivators participate corepressor-coactivator interacition. (A) N-CoR and SMRT interact with ACTR in a GST pull-down assay. $ACTR_{400-1000}$ was expressed as GST fusion in BL21 (DE3) bacteria. $Myc-N-CoR_{759-2453}$, pCMX-N-CoR, and pCMX-SMRT (SMRT clone with full length 1495 amino acids, Chen and Evans, 1995) were used to generated ^{35}S-met labeled protein. (B) ACTR, SRC-1 and GRIP1 interact with N-CoR in the mammalian two-hybrid assay. The interaction between GAL4 fusion and VP16 fusion was measured through a mammalian two-hybrid assay on a 5GAL4Luc3 reporter gene in CV1 cells. A CMV-β-gal internal control plasmid was used to normalize the luciferase values for transfection efficiency. Means $\pm$ st.dev. are shown.

11

Figure 4

A

10% Input	+	−	−	−	−
GST-ACTR400-1000	−	+	+	+	+
N-CoR759-2453	−	−	−	+	+
T3	−	−	+	−	+

B

Gal4-ACTR 400-1000	+	+	+	+	+	+	+	+
VP16	+	−	−	−	+	−	−	−
VP16-TRβ (WT)	−	+	−	−	−	+	−	−
VP16-TRβ (L454R)	−	−	+	−	−	−	+	−
VP16-TRβ (E457K)	−	−	−	+	−	−	−	+
		vec				N-CoR		

C

Gal4-ACTR	+	+	+	+	+	+	+	+	+	+	+	+
VP16-N-CoR759-2453	−	+	−	+	−	+	−	+	−	+	−	+
T3	−	−	−	−	+	+	−	−	+	+	−	−	+	+
TRβ		vec		WT			L454R			E457K		
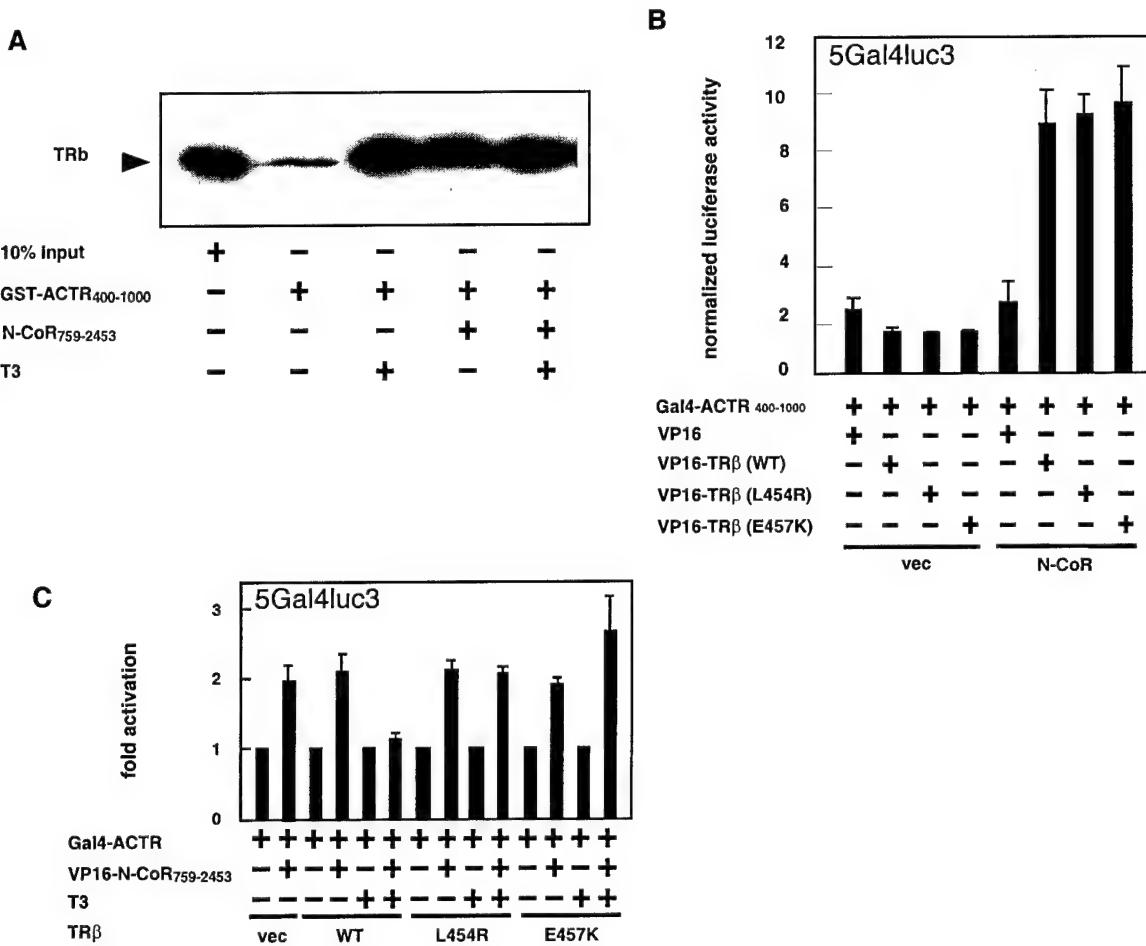
Figure 4. N-CoR, ATCR and TR form a trimeric complex
(A) N-CoR promotes ACTR/TR interaction in a GST pull-down assay. GST-ACTR400-1000 was isolated using glutathione-conjugated sepharose beads and was used to pull down radio-labeled TRβ. *In vitro* translated non-labeled N-CoR759-2453 or equal amount of reticulocyte lysate was added to each assay. (B) N-CoR promotes ACTR/TR interaction in the mammalian two-hybrid assay. Gal4-ACTR400-1000 and VP16-TR was used in transfection of CV-1 cells. pcDNA3-N-CoR or equivalent molar amount of pcDNA3 vector was added to each assay. (C) Liganded TR destabilizes N-CoR/ACTR interaciton in the mammalian two-hybrid assay. CV1 cells were transfected with 5xGa4luc3 reporter, CMV-β-gal internal control vector and the plasmids indicated as indicated. Empty VP16 and pcDNA3 vectors are used to assure that each assay contain equivalent molar amount of VP16 and CMV promoters.

Figure 5

A
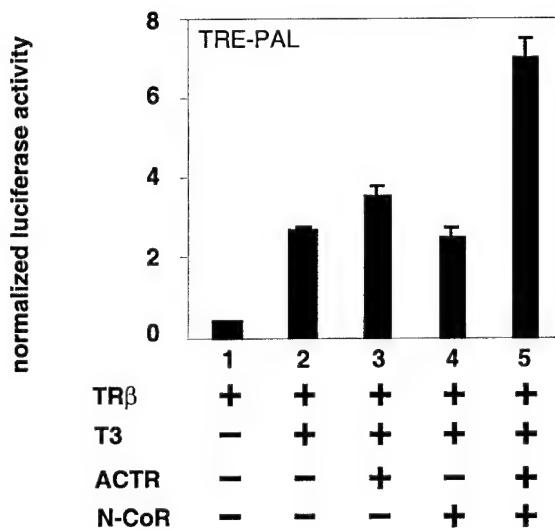
B
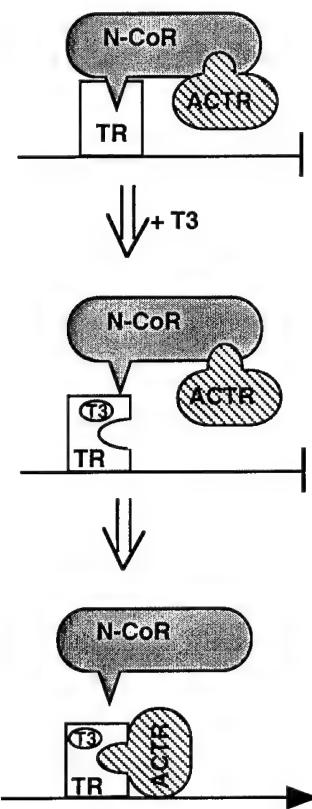
Figure 5. N-CoR and ACTR cooridnate in thyroid hormone transcriptional activation.
(A) N-CoR potentiates transcriptional activation of TR/ATR complex. TRE-pal-luciferase reporter, CMV-β-gal, pcDNA3-TRβ, pcDNA3-ACTR, and pCMX-N-CoR were co-transfected into CV-1 cells. EGFP-pcDNA3 was added to assays to assure that each assay contains equal amount of CMV promoters. After 30 hours incubation, T3 (10^{-7}M) was added and cells were incubated for additional 12 hours. (B) A model to explain how N-CoR and ACTR coordinate and regulate thyroid hormone action.

Figure 6

A

Gal4   +   −   +   −
Gal4-N-CoR 759-2453   −   +   −   +
           vec   ACTR 400-1000

B

HADC4 ►

input   GST   GST-RD3   GST-RD3
         lysate   ACTR

C

N-CoR

RD3

HDAC4   AC TR
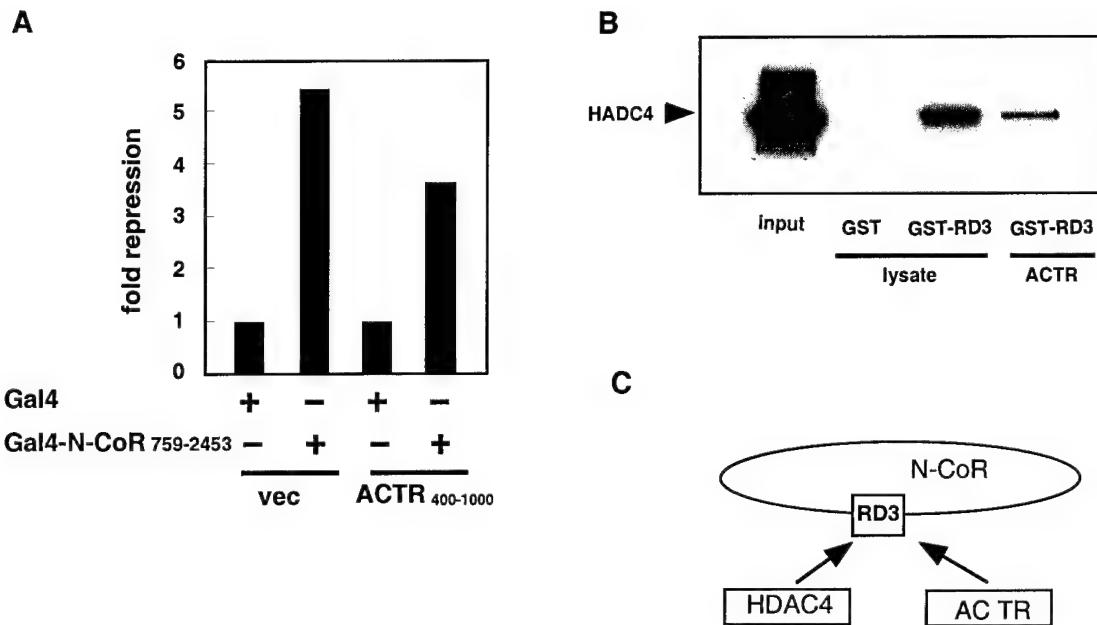
Figure 6. ACTR modulates N-CoR mediated transcriptional repression.
(A) ACTR partially reverses the repression funciton of Gal4-N-CoR759-2453. CV1 cells were transiently transfected with 5XGal4luc3 reporter, CMV-β-gal, and the plasmids as indicated. (B) ACTR destablizes the interaction between HDAC4 and RD3. N-CoR domain RD3 (aa 1017-1461) was expressed as a GST fusion protein and isolated using glutathione-conjugated beads. ^{35}S-HDAC4 and non-labeled ACTR were made using pCMX-Gal4-HDAC4, pcDNA3-ACTR in the TNT system (Promega). Bead-coupled GST or GST-RD3 (2 μg each) were incubated with ACTR or equivalent amount of reticulocyte lysate for 12 hours before the addition of ^{35}S-HDAC4, which was then added for additional 1 hour incubation. Input: 10%.

14

Key Research Accomplishments

1. Identification of protein-protein interaction surfaces on N-CoR using phage display.

2. Discovered that N-CoR interacts directly with ACTR in vitro and in vivo.

3. Characterization of the interaction surface on N-CoR and ACTR.

4. Identified that N-CoR, ACTR and TR form a trimeric complex in target cells.

5. Identified that N-CoR potentiates transcriptional activation of TR/ACTR complex.

6. Identified that ACTR reverses N-CoR-mediated transcriptional repression.

Reportable Outcomes

Manuscripts:

Xiaolin Li, Erin A. Kimbrel, Daniel J. Kenan, and Donald P. McDonnell.
N-CoR, ACTR, and TR form a trimeric complex within target cells physically linking the processes of transcriptional activation and repression. Submitted.

Conclusions

Progesterone receptor transcriptional activity is modulated by corepressors and coactivators. In the first year of this research, we have identified direct interaction between corepressor N-CoR with coactivator ACTR. The significance of the finding was supported by the observation that that N-CoR contributes to transcriptional activation by recruiting ACTR to nuclear receptor TR. In addition, ACTR appears to partially reverse the transcriptional repression of N-CoR by interfering with N-CoR/HDAC4 interaction. Thus, the link between transcriptional repression and activation is more closely integrated than previously thought. We will next test how corepressor and coactivator coordinate in regulating progesterone action in normal cells and breast cancer cells.

References

1. Osborne, C.K., *Steroid hormone receptors in breast cancer management.* Breast Cancer Res Treat, 1998. **51**(3): p. 227-38.

2. Shibata, H., *et al., Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action.* Recent Prog Horm Res, 1997. **52**: p. 141-64.

3. Jackson, T.A., *et al., The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT.* Mol Endocrinol, 1997. **11**(6): p. 693-705.

4. Wagner, B.L., *et al., The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor.* Mol Cell Biol, 1998. **18**(3): p. 1369-78.

5. Lavinsky, R.M., *et al., Diverse signaling pathways modulate nuclear receptor recruitment of N- CoR and SMRT complexes.* Proc Natl Acad Sci U S A, 1998. **95**(6): p. 2920-5.

6. Anzick, S.L., *et al., AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer.* Science, 1997. **277**(5328): p. 965-8.

7. Chen, H., *et al., Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300.* Cell, 1997. **90**(3): p. 569-80.

8. Horlein, A.J., *et al., Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor.* Nature, 1995. **377**(6548): p. 397-404.

9. Zhang, J., *et al., Proteasomal regulation of nuclear receptor corepressor-mediated repression.* Genes Dev, 1998. **12**(12): p. 1775-80.

10. Feng, W., *et al., Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors.* Science, 1998. **280**(5370): p. 1747-9.

11. Huang, E.Y., *et al., Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway.* Genes Dev, 2000. **14**(1): p. 45-54.

12. Chang, C., *et al., Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors alpha and beta.* Mol Cell Biol, 1999. **19**(12): p. 8226-39.